Both models of PlexinD1 function reveal some intriguing aspects. Even though vascular patterning is severely disturbed, the mutants develop surprisingly far. In the mouse, the defects in the large coronary vessels and in heart structure are what causes perinatal death. In the fish, defects in the trunk are largely restricted to the ventral somite areas. Can the loss of PlexinD1 function be compensated by other semaphorin/neuropilin/plexin complexes in the dorsal region? Importantly, as indicated above, vascular expression of NRP2 is predominantly confined to lymphatic endothelium at E13 already, while NRP1 continues to be expressed in blood vascular endothelium. It can therefore be expected that double mutants lacking both PlxnD1 and NRP1 will exhibit a phenotype different from that of PlxnD1/NRP2 double deficient embryos. This can also be inferred from the finding that sema3C but not sema3A can bind to PlxnD1/NRP2 receptor complexes.

The PlexinD1 functions described are caused by what has been termed long-range effects of secreted semaphorins. Can we expect that membrane-bound semaphorins mediate short-range effects elicited upon cell-to-cell interaction? Could such interactions mediate neuropilin-independent functions of PlexinD1? In the present studies, semaphorin activity has been suggested to be of a repelling nature. There are, however, instances in which semaphorins exhibit chemoattractant functions. Furthermore, the response to certain semaphorins is modulated also by the absence or presence of receptor tyrosine kinases with the respective plexin receptor; among those is VEGFR2.

Finally, not only is PlexinD1 a candidate gene for Möbius syndrome, but due to the cardiac phenotype of the mouse PlexinD1 mutant, it will also be interesting to elucidate its role in congenital heart disease and other clinical settings.

This work thus reinforces the question of whether guidance cues are generally conserved between structurally if not functionally similar entities such as the nervous system and the vasculature. In addition, where both nerves and vessels are spatially aligned, molecular interactions within diffusion distances between them are possible and should be investigated. All in all, the discovery of an essential role for the endothelial PlexinD1 has revealed new avenues for investigation into an already quite intricate web of factors involved in the spatial organization of vascular and conversely also neural networks.

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More Than Cell Death: Caspases and Caspase Inhibitors on the Move

It is becoming clear that "apoptotic" caspases can effect cellular processes other than cell death. A recent paper in *Cell* points to a novel role of the *Drosophila* caspase inhibitor DIAP1 as a determinant of cell migration.

It's an emerging theme that proteins with well-established biochemical functions fulfill additional, surprising roles in other contexts. An important example is β-catenin, first identified as a major component of adherens junctions, but subsequently found to be of key importance in Wnt-induced transcription. Similarly, several cell cycle-regulatory proteins appear to play broader cell cycle-independent functions during development and differentiation. Caspases until recently were thought to be solely effectors of apoptosis. However, a number of reports have implicated these enzymes in other separate processes including differentiation (Newton and Strasser, 2003; Okuyama et al., 2004, and references therein). A paper in the July 9 issue of *Cell* now uncovers a connection between a caspase inhibitor, the *Drosophila* DIAP1 protein, and control of cell migration.

Using an elegant genetic approach, aimed at identifying second site loci that revert the phenotype caused by loss of Rac1 function in ovary cell border migration, Geisbrecht and Montell (2004) show that lack of Rac1 function can be compensated, besides by an increase in G-actin production, by overexpression of DIAP1, an endogenous caspase inhibitor. DIAP1 is a member of an evolutionary conserved family of proteins that contain BIR (caspase and IAP antagonist interacting) domains. Interestingly, the function of BIR domain containing proteins in yeast and *C. elegans* was already previously connected with control of cytokinesis and the cytoskeleton. Various Diap-1 (thread) mutants have been previously characterized, which map either in the BIR or RING finger domains of the protein and show reduced,

or in some cases enhanced, ability to prevent cell death induced by IAP antagonists (see, for instance, Goyal et al., 2000). Geisbrecht and Montell show that the BIR mutants of DIAP1 have defects in border cell migration, while a DIAP1 mutant in the RING finger does not. Because the RING finger mutant is also impaired in inhibiting apoptosis, this result shows that DIAP1's ability to prevent apoptosis and to allow border cell migration can be segregated. This conclusion is further supported by the fact that DIAP2 is slightly better than DIAP1 at rescuing the border cell migration defect; to date, DIAP2 has been the "runt of the litter" and is relatively ineffective at inhibiting caspases and preventing cell death (Hay, 2000).

Importantly, the authors found that the general caspase inhibitor p35 is incapable of suppressing the RacN17-induced defects in cell migration. This points to a possible involvement of Dronc, the *Drosophila* homolog of mammalian caspase 9, which can be inhibited by DIAP1 but not p35. And indeed, overexpression of dominant-negative Dronc is also sufficient to rescue the RacN17-induced defects. While these results strongly suggest that the role of DIAP1 in this context is to limit Dronc activity, such conclusion needs to be taken with some caution. In fact, although DIAP2 does not appear to be a very effective inhibitor of Dronc (Hawkins et al., 2000), it seems to be more effective than DIAP1 in rescuing the RacN17-induced defect.

As is often the case, many questions are raised by important novel findings such as these. In fact, the mechanism whereby increased DIAP1 rescues the Rac1 deficiency remains to be clarified. The authors show that in S2-transfected cells, DIAP1 can associate with the Rac1 protein and that this protein also associates with profilin, a determinant of free G-actin. However, whether or not all three proteins can be present in the same complex is not known, nor whether Dronc is also present in this complex and/or whether Dronc controls DIAP1/Rac1 complex formation. Since DIAP1 associates with Rac1 irrespective of its GDP- or GTP-bound state, the functional meaning of this association remains also to be seen. Thus, two scenarios can be envisaged. In one, the function of DIAP1 on migration is still mediated by negative regulation of a specific caspase, such as Dronc. In the other, DIAP1 has an independent function on actin and cell migration, with Dronc negatively regulating DIAP1 activity.

Intriguingly, both DIAP1 and DIAP2 have been shown to interact with thick veins (Oeda et al., 1998), a BMP family receptor, that might play a role in border cell migration. Another potential cross-talk is suggested by the fact that *ras* which has also been shown to play a role in border cell migration can inhibit the proapoptotic activity of the IAP antagonist Hid (Bergmann et al., 1998). Thus *ras* signaling might lower the levels of active Hid that could regulate the activity of DIAP1.

More generally, as mentioned at the beginning, an exciting line of work for future studies is the unexpected high degree of interconnection between molecules until recently thought to be involved in entirely separate functions. While detailed understanding of biological processes forces us to think of them as distinct events, ultimately it will be essential to understand how their integration is achieved, with a number of molecules, such as the ones that we have discussed, being directly implicated in this integration.

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Making Tubes: Step by Step

Branched hollow tubes form the architectural basis of many mammalian organs. The growth factor HGF/SF and its receptor, the Met receptor tyrosine kinase, stimulate epithelial cells to undergo tubulogenesis in vitro. In this issue of *Developmental Cell*, O'Brien et al. (2004) look at temporal regulation and the role of two HGF/SF effectors, the ERK 1/2 MAP kinases and matrix metalloproteases, in this process.

The reorganization of epithelial cells into branched tubular structures represents the structural basis for the formation of a variety of mammalian organs such as the mammary gland, liver, lung, and kidney. In mammalian organ formation, these tubular structures arise from buds of epithelial cells which undergo changes in cellular polarity and adhesion (so-called epithelial-mesenchymal transition, EMT; Thiery, 2003), migrate, divide, and finally redifferentiate to form polarized branched hollow tubules with organ-specific functions. This process requires precise control over the cellular cytoskeleton and adhesion, cell division, and apoptosis, and also active alteration of the cellular environment (Rosário and Birchmeier, 2003). Unsurprisingly, in vivo tubulogenesis